The Minimal "Chlorine Death Points" of Bacteria

I. Vegetative Forms

FRED O. TONNEY, M.D., FRANK E. GREER,
and
T. F. DANFORTH, D.V.M.

Bureau of Laboratories and Research, Department of Health, Chicago, Ill.

ALTHOUGH chlorine gas is universally used in the treatment of water supplies, and its derivatives are finding an ever increasing field of usefulness in the disinfection of milk and beverage bottles, dishes, and utensils of various kinds, little is known of the actual or relative amounts of chlorine required to kill specific species of organisms under given conditions. The amount of available chlorine used in practical chlorination has been arrived at empirically or indirectly, and in many instances is probably far in excess of the amount actually needed to destroy the pathogen under consideration. The amount of chlorine required to obtain satisfactory results varies with many factors, and especially with the kind of fluid or material to be sterilized.

Little, if any, work has been done to determine the least amount of chlorine required to kill known numbers of known species of bacteria under fixed conditions that admit of comparison of their relative resistance.

It has not been determined definitely what organism may be considered a safe index of effective chlorine treatment or whether a given organism can be used as an index under different conditions. *B. coli* has been almost universally accepted as the index of safety in the treatment of water supplies. Its acceptance, however, has been based largely on theoretical considerations and on the analogy of its greater resistance to heat and longer survival than the common pathogens which may be present in water.

For these reasons it was considered desirable to study the effect of chlorine on specific bacteria under controlled conditions to determine, if possible, the minimal dosage of free chlorine required to kill specific species of bacteria, to ascertain their relative resistance to free chlorine and, on the basis of these factors, to select, if possible, an organism that could be used as a safe index of effective chlorine disinfection.

In this article the methods used and results obtained with vegetative forms of bacteria are presented.

The methods of preparing the materials for the determinations were as follows:

The chlorine solution used was prepared by adding gaseous chlorine to distilled water until a final concentration of 60 p.p.m. of residual free chlorine was obtained. This served as a stock solution and was stored in a brown bottle in an icebox. At frequent intervals the free chlorine content of the solution was determined, and approximately every two weeks during the experiment a new stock solution was prepared.

The organisms tested included the common intestinal forms, and other pathogenic and non-pathogenic organisms, numbering in all 235 strains.

The intestinal organisms and *B. proteus, B. prodigiosus* and *B. pyocyaneus* were grown on agar slants. *Cl. welchii* was grown in glucose broth under anaerobic conditions. The streptococci and pneumococci were grown either in glucose broth or in a special streptococcus broth. The staphylococci were grown in glucose broth and the diphtheria organisms were grown in glucose broth, ascites fluid or serum glucose broth.

The cultures were centrifuged, washed, and resuspended three times in sterile distilled water. They were then diluted in sterile distilled water until a suitable number of organisms per c.c. were present.

The exposures to chlorine were made in sterile distilled water as a menstruum. One hundred and fifty c.c. of distilled water were placed in Pyrex Erlenmeyer flasks, and the contents and flasks were sterilized in an Arnold sterilizer. After the flasks had cooled to room temperature, 50 c.c. of the water in each flask were removed and the chlorine absorbing properties determined. Those flasks containing water showing any appreciable absorption were discarded. The remaining flasks were used for the experiments. The pH value of the water used ranged from 6.4 to 7.2. It is probable that the elimination of the water showing chlorine absorption also eliminated water of low or high pH value.

The tests were carried out by adding approximately 100 to 300 organisms per c.c. to the contents of each of two flasks. From one flask 1 c.c. was planted to determine the number of organisms present. Then a definite amount of free chlorine was added to each of the flasks. One flask was immediately subjected to chemical analysis to determine the amount of residual chlorine present. The other flask was

used for determining the rate of destruction of the organisms and the time required to kill. To do this 1 c.c. of the contents was plated at intervals of 8–10 seconds, 15 seconds, 30 seconds, 45 seconds and 60 seconds. The nutrient agar plates were poured immediately after transferring each 1 c.c. portion from the flasks. These plates were incubated for 48 hours at 37° C. At the end of this time the plates were counted and the rate of destruction and time required to kill the organisms present recorded. It was found that 48-hour incubation was necessary, because in many instances numerous colonies appeared in 48 hours on plates which in 24 hours had no visible colonies. For the organisms that would not grow on plain agar, slight variations of the above procedure were used. With such organisms it was not possible to establish the rate of destruction by chlorine, as no satisfactory means of counting them was available, either before or after the addition of the germicide. It was only possible to determine the length of time required to kill all of the organisms. For this purpose the contents of the flasks were streaked or planted in the type of medium in which the organisms were originally grown. The experiments were carried out at temperatures ranging from room temperature to within a few degrees of the freezing point of water.

RESULTS

The results are shown in Table I. It will be noted that all strains of *B. typhosus, B. paratyphosus A, B. paratyphosus B, B. enteriditis, B. dysenteriae, B. suipestifer, B. proteus, B. prodigiosus,* and *B. pyocyaneus* were killed in 15 seconds by a dosage of 0.1 p.p.m. of free chlorine.

Many of the strains of streptococci of both hemolytic and fecal types were also killed by this amount of chlorine, as were all the strains of *Cl. welchii* (vegetative cells) and most of the strains of *C. diphtheriae.* In some instances slightly longer periods of time, i.e., 30 to 45 seconds, were required to kill all the organisms at the concentration of 0.1 p.p.m.

A few strains of pneumococci were slightly more resistant to chlorine than most of the other organisms. Four out of nine strains were killed by 0.1 p.p.m. of chlorine in 15 seconds. The remaining strains were killed by 0.2 p.p.m. of chlorine in less than 1 minute. The strains of *C. diphtheriae* not killed by 0.1 p.p.m. of chlorine were killed by 0.15 p.p.m. in 15 to 30 seconds.

Most of the strains of streptococci not killed by 0.1 p.p.m. of chlorine were killed by 0.15 or 0.20 p.p.m., although three strains were not killed until exposed to 0.25 p.p.m. of chlorine for 15 seconds to 30

TABLE I

DOSAGE OF FREE CHLORINE REQUIRED TO KILL VEGETATIVE CELLS OF BACTERIA IN 15–30 SECONDS

Chlorine p.p.m.	Species	No. of Strains	Species	No. of Strains
0.10	*B. typhosum*	21	*B. pyocyaneus*	6
	B. paratyphosum A	6	*C. diphtheriae*	27
	B. paratyphosum B	6	*Achromo bacterium viscosum*	3
	B. dysenteriae	8	*Strep. scarlatinae*	14
	B. enteriditis	4	*Strep. fecalis*	11
	B. proteus	2	*B. suipestifer*	8
	Cl. welchii	8	*B. prodigiosus*	6
	Bruc. melitensis	1	*Bruc. abortus*	1
	Staph. albus	4	*Staph. aureus*	4
	Pneumococcus	4	*Strep. hemolyticus*	11
0.15	*C. diphtheriae*	12	*Strep. morbilli*	2
	Strep. scarlatinae	6	*Staph. albus*	4
	Strep. fecalis	2	*Pneumococcus*	5
	Staph. aureus	4	*B. coli* (fecal)	9
	Strep. hemolyticus	10		
0.20	*Pneumococcus*	4	*B. coli*	10
0.25	*Strep. hemolyticus*	3	*B. coli* (fecal)	9
			Total Strains	235

seconds. These latter strains were of the hemolytic variety. Two were isolated from milk and one came from the Boston epidemic of streptococcus sore throat of 1911. It seems probable that the apparently higher resistance of these cocci to chlorine was due to the larger inoculum found necessary to obtain consistent growths. Considerably heavier inoculations of the streptococci were required than in the case of other organisms.

B. coli proved on the whole to be more resistant to free chlorine than the other organisms studied. None of the strains showed any appreciable reduction in number when exposed to 0.1 p.p.m. of chlorine. Nine strains were killed by 0.15 p.p.m. of chlorine, ten strains required 0.2 p.p.m., and nine required exposure for 15 seconds to 0.25 p.p.m. of chlorine for their complete destruction.

SUMMARY AND CONCLUSIONS

From these results it will be seen that a large majority of the vegetative types of organisms considered to be of sanitary importance in connection with water supplies, milk supplies, dish washing, bottle washing, and general disinfection, whether of intestinal or respiratory origin, are killed in a few seconds by rather small doses of free chlorine, when exposed in a suspension containing no organic matter or other substances that react with or absorb the chlorine. This was

found to be true for temperature ranges varying from room temperature to within a few degrees of the freezing point of water.

The amount of chlorine required to kill most of the intestinal pathogens studied under these conditions was 0.1 p.p.m., with the greatest destruction of the organisms occurring within the first 15 seconds of exposure. The same amount of chlorine destroyed most of the pathogenic types of respiratory organisms tested.

In each group, however, there were individual strains which were more resistant to chlorine than the others, requiring more than 0.1 p.p.m. to accomplish their destruction. In the intestinal group of organisms, the most resistant type encountered was *B. coli*. In the group of respiratory origin, three strains of hemolytic streptococci, out of 24 strains tested, required larger quantities of free chlorine for their complete destruction. All the resistant strains of streptococci were killed by 0.25 p.p.m. in 15 to 30 seconds. It is probable that the apparently higher resistance of the streptococcic strains is due to the larger number of organisms used in order to secure consistent growths.

From the standpoint of resistance to chlorine, *B. coli* stands out among the organisms tested as the most suitable for use as an index of the effectiveness of chlorine disinfection. The requisites of such an index organism are: (1) that it be as resistant as, or somewhat more resistant to, free chlorine than the pathogenic organisms which are to be destroyed; (2) that it grow readily on simple media; (3) that it be readily recognizable by simple routine tests. From the standpoint of availability, rapidity of growth, and ready detection, *B. coli* offers superior advantages as an index organism, and it is our feeling that its consistent absence in a menstruum after chlorine disinfection is valuable evidence of the destruction of the pathogens here studied.

On the whole, the experiments appear to furnish a satisfactory theoretical basis for the current practice of relying on the consistent destruction of *B. coli* in water as a criterion of effective chlorination, and that they may also justify a more general application of the same criterion to other type phases of chlorine disinfection now being developed, such as the washing of milk bottles and equipment and the washing of dishes and eating utensils.